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Serum C-peptide concentrations poorly phenotype type 2 diabetic end-stage renal disease patients

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Background. A homogeneous patient population is necessary to identify genetic factors that regulate complex disease pathogenesis. In this study, we evaluated clinical and biochemical phenotyping criteria for type 2 diabetes in end-stage renal disease (ESRD) probands of families in which nephropathy is clustered. C-peptide concentrations accurately discriminate type 1 from type 2 diabetic patients with normal renal function, but have not been extensively evaluated in ESRD patients. We hypothesized that C-peptide concentrations may not accurately reflect insulin synthesis in ESRD subjects, since the kidney is the major site of C-peptide catabolism and would poorly correlate with accepted clinical criteria used to classify diabetics as types 1 and 2.

Methods. Consenting diabetic ESRD patients ($N = 341$) from northeastern Ohio were enrolled. Clinical history was obtained by questionnaire, and predialysis blood samples were collected for C-peptide levels from subjects with at least one living diabetic sibling ($N = 127$, 48% males, 59% African Americans).

Results. Using clinical criteria, 79% of the study population were categorized as type 1 (10%) or type 2 diabetics (69%), while 21% of diabetic ESRD patients could not be classified. In contrast, 98% of the patients were classified as type 2 diabetics when stratified by C-peptide concentrations using criteria derived from the Diabetes Control and Complications Trial Research Group (DCCT) and UREMIDIAB studies. Categorization was concordant in only 70% of ESRD probands when C-peptide concentration and clinical classification algorithms were compared. Using clinical phenotyping criteria as the standard for comparison, C-peptide concentrations classified diabetic ESRD patients with 100% sensitivity, but only 5% specificity. The mean C-peptide concentrations were similar in diabetic ESRD patients (3.2 ± 1.9 nmol/L) and nondiabetic ESRD subjects (3.5 ± 1.7 nmol/L, $N = 30$, P = NS), but were 2.5-fold higher compared with diabetic siblings (1.3 ± 0.7 nmol/L, $N = 30$, $P < 0.05$) with normal renal function and were indistinguishable between type 1 and type 2 diabetics. Although 10% of the diabetic ESRD study population was classified as type 1 diabetics using clinical criteria, only 1.5% of these patients had C-peptide levels less than 0.20 nmol/L, the standard cut-off used to discriminate type 1 from type 2 diabetes in patients with normal renal function. However, the criteria of C-peptide concentrations >0.50 nmol/L and diabetes onset in patients who are more than 38 years old identify type 2 diabetes with a 97% positive predictive value in our ESRD population.

Conclusions. Accepted clinical criteria, used to discriminate type 1 and type 2 diabetes, failed to classify a significant proportion of diabetic ESRD patients. In contrast to previous reports, C-peptide levels were elevated in the majority of type 1 ESRD diabetic patients and did not improve the power of clinical parameters to separate them from type 2 diabetic or nondiabetic ESRD subjects. Accurate classification of diabetic ESRD patients for genetic epidemiological studies requires both clinical and biochemical criteria, which may differ from norms used in diabetic populations with normal renal function.

Classification of diabetics as type 1 (very low or absent endogenous insulin secretion) or type 2 (significant pancreatic insulin production with peripheral insulin resistance) remains imprecise, although large, well-designed studies have validated both clinical and biochemical criteria in non-end-stage renal disease (ESRD) patients [1–4]. Intermediate diabetic phenotypes have recently been recognized, further complicating categorization. For example, 10 to 15% of subjects with clinical features suggestive of type 2 diabetes have slowly progressive, late onset, autoimmune injury to pancreatic β cells [5, 6], which results in a lack of insulin production. Conversely, some patients with idiopathic type 1 diabetes do not have an autoimmune disorder [7].

Classification of diabetes is important for several reasons. First, appropriate categorization can guide therapy for diabetic patients since insulin treatment in some type 2 patients may result in hyperinsulinemia with adverse effects [8]. In addition, novel, mechanism-based thera-

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pies for diabetes have been developed, such as immunomodulators and antiviral agents for type 1 diabetics [9–11] or troglitazone for type 2 diabetics [12]. Second, imprecise classification of diabetes has generated conflicting, epidemiological descriptions of diabetic nephropathy. In the past, some authors have suggested that type 1 diabetics had an increased chance of developing ESRD [13], whereas more recent studies demonstrated a similar risk for nephropathy in both type 1 and type 2 diabetics [14]. Finally, accurate phenotypes are critical for identifying chromosomal loci, which contain genes that cause complex diseases [15]. Although genetic factors regulate, in part, diabetic nephropathy pathogenesis [recently reviewed in 16–18], reports of nephropathy susceptibility loci have been conflicting and not replicated in different patient populations. Incomplete phenotyping, as well as study design (for example, case control vs. sibling pair), undoubtedly increased heterogeneity within the study populations [17–20] and has confounded reproducible identification of diabetic nephropathy loci. Molecular mechanisms of type 1 and type 2 diabetes are different, suggesting that different genetic pathways may regulate nephropathy susceptibility in type 1 and type 2 diabetics independently. As a result, accurate classification of type 1 versus type 2 diabetic ESRD patients should improve the likelihood of identifying diabetic nephropathy genes.

We are currently conducting a large multicenter study, using a family based strategy, to identify susceptibility genes for type 2 diabetic nephropathy. In other reports, C-peptide concentrations combined with clinical criteria have been used to identify type 2 diabetics, with and without ESRD [1, 2, 21]. However, the value of C-peptide concentrations in classifying diabetic ESRD patients has not been extensively evaluated. Since the kidney is the major site for C-peptide catabolism and excretion [22–26], we hypothesized that C-peptide concentrations may not improve the power of clinical criteria to categorize ESRD patients, in contrast to patients with normal renal function, as type 1 and type 2 diabetics.

METHODS

Study population

The study population was selected from prevalent ESRD patients in eight hemodialysis units in metropolitan Cleveland (OH, USA). From the population screened ($N = 1237$), 915 completed a screening questionnaire to obtain medical history and family history of renal disease. Of these, 341 patients (37.3%) had a diagnosis of diabetes on their Health Care Financing Administration (HCFA) 2728 forms and a long-standing history of diabetes therapy with diet, insulin, and/or oral hypoglycemic agents. From this group, 127 ESRD patients (13.9%) with a family history of diabetes and at least one living diabetic sibling have been phenotyped and genotyped. To phenotype these patients, the following information was obtained: age, age at diabetes onset, age at dialysis initiation, duration of kidney disease, weight and height at 18 years of age and at the time of the interview, maximum weight during life, onset and duration of diabetes treatment with diet and/or oral hypoglycemic drugs and/or insulin, history of diabetic coma, and/or diabetic ketoacidosis (DKA). Medical records, when available, were used to confirm the interview data. Simultaneous serum glucose and C-peptide levels were determined for each patient from blood samples obtained at the beginning of a dialysis treatment (discussed later in this article). Body mass index was calculated as patient weight (in kilograms) divided by the square of patient height (in meters). The Institutional Review Boards of the MetroHealth System, University Hospitals of Cleveland, and the Cleveland Clinic Foundation approved this protocol.

Table 1. Classification criteria

Type 1 diabetes
1. Age of onset <25 years [21, 29, 30] and one of the following criteria:
   a. History of diabetic ketoacidosis [21, 29, 30, 33];
   b. Treatment with insulin only and/or insulin therapy initiated less than one year after diabetes diagnosis [21, 29, 33];
   c. Weight at diagnosis and/or maximal weight <105% of the ideal body weight [21, 27, 29, 30].

Type 2 diabetes
1. Onset of diabetes after 40 years of age [2, 21, 30, 31], no history of diabetic ketoacidosis [4, 29], and one of these additional criteria:
   a. Weight at diagnosis and/or maximum weight >115% of ideal body weight [21, 28–30];
   b. No consistent insulin therapy during the first two years after diabetes diagnosis [21, 29, 31].
2. Both (1a) and (1b) if diabetes onset occurred between 30 and 40 years [34].

Unclassified diabetics: patients not categorized by the above criteria

Clinical diabetic phenotyping criteria

We developed clinical criteria to classify ESRD patients as type 1 or type 2 diabetics using the following reports: (*1*) WHO and American Diabetes Association 1997 guidelines [27, 28], (*2*) studies of diabetic ESRD patients (Michigan Kidney Registry [29] and UREMID-IAB Study [30]), (*3*) studies of diabetics with normal [2, 31] and abnormal glomerular filtration rate (GFR) [21] who were phenotyped using clinical parameters and C-peptide concentrations, and (*4*) studies of genetic susceptibility for type 2 diabetes [1, 17, 32]. Table 1 lists the criteria we used to classify enrolled ESRD patients as type 1 or 2 diabetics.

Serum glucose and C-peptide assays

The Core Laboratory of the General Clinical Research Center of Case Western Reserve University measured

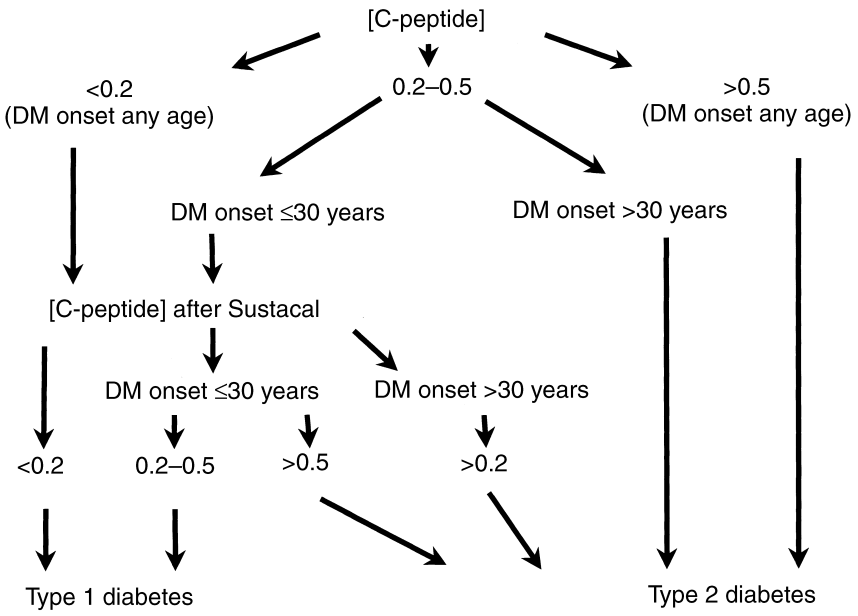

Fig. 1. An algorithm to classify diabetes according to C-peptide concentrations. As described in the **Methods** section, C-peptide concentrations were assayed in a serum sample obtained at the initiation of dialysis, and diabetes was classified as type 1 or type 2. C-peptide concentrations are expressed as nmol/L. Prior to classification, serum C-peptide levels were repeated after overnight fasting and Sustacal stimulation in study participants, who had random C-peptide concentrations <0.5 nmol/L and diabetes onset prior to an age of 30, or in subjects of any age with C-peptide concentrations <0.2 nmol/L. No additional tests were obtained in study participants, who had serum C-peptide concentrations >0.5 nmol/L or in subjects with C-peptide concentrations from 0.2 to 0.5 nmol/L but in whom diabetes onset occurred after 30 years of age. Abbreviations are: [C-peptide], C-peptide concentrations; DM, diabetes mellitus; years, years of age.

serum glucose and C-peptide concentrations. Glucose was assayed using glucose-oxidase, and C-peptide concentrations were determined using a standard double-antibody radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). For C-peptide determinations, blood was collected by venipuncture in serum separator (red-gray top, no anticoagulant) tubes, and the cells were separated by centrifugation. Standard concentrations of serum-based, human C-peptide, and patient sera were mixed with [^{125}I]C-peptide and rabbit anti–C-peptide antiserum for four hours at room temperature. A precipitating solution, containing goat anti-rabbit γ-globulin and polyethylene glycol, was used to separate bound from free [^{125}I]C-peptide. The antibody-bound fraction was precipitated and counted in a γ-counter. Patient sample concentrations were determined from a calibration curve, generated from the standards. Coefficients of variation for intra-assay and interassay variability were 3.9 ± 1.1% and 4.7 ± 2.4%, respectively. The assay detection limit was approximately 0.07 nmol/L.

Sustacal-stimulated C-peptide concentrations

After an eight-hour fast (from midnight), baseline glucose and C-peptide concentrations were determined. Sustacal (6 cc/kg, maximum 360 cc) was then administered orally over 10 minutes. After 90 minutes, samples for stimulated glucose and C-peptide measurements were obtained.

C-peptide phenotyping criteria

In contrast to most published studies, which measure basal and post-stimulation C-peptide concentrations to document absence of endogenous insulin production [1, 2], our goal was to identify patients with type 2 diabetes (Fig. 1). Since insulin synthesis is maintained, even long after apparent disease onset in type 2 diabetics [35], we obtained predialysis blood samples (at 7 a.m. for first shift, 12 p.m. for second shift, and 4 p.m. for third shift subjects) for serum glucose and C-peptide concentrations. Diabetic ESRD patients were classified as type 1 or type 2 diabetics according their C-peptide concentrations using the Diabetes Control and Complications Trial Research Group (DCCT) algorithm [2] with the following modifications:

Pre-HD C-peptide >0.50 nmol/L. These diabetic ESRD patients were classified as type 2 diabetics without a Sustacal-stimulation test since in the DCCT study, only type 2 diabetics had basal or stimulated C-peptide concentrations >0.50 nmol/L (Fig. 1).

Pre-HD C-peptide value between 0.20 nmol/L and 0.50 nmol/L. Using DCCT criteria [2], a Sustacal stimulation test was performed if diabetes onset occurred before the age of 30 (Fig. 1). ESRD patients with Sustacal-stimulated C-peptide concentrations >0.50 nmol/L were classified as type 2 diabetics. Patients with stimulated C-peptide concentrations <0.50 nmol/L were classified as type 1 diabetics. If diabetes onset was after 30 years of age, patients were categorized as type 2 diabetics without performing a Sustacal-stimulation test [2].

Pre-HD C-peptide value <0.20 nmol/L. Sustacal-stimulation tests were performed in all patients with pre-hemodialysis C-peptide concentrations <0.20 nmol/L (0.6 ng/L; Fig. 1). If stimulated C-peptide concentrations remained <0.20 nmol/L, subjects were considered type 1 diabetics regardless of age of diabetes onset. Patients were classified as type 2 diabetics if the age of diabetes onset was after the patient was 30 years of age, and the stimulated C-peptide concentrations were >0.20 nmol/L.

Table 2. Clinical characteristics and C-peptide levels of the study population ($N = 127$)

Parameter	Mean	SD	Minimum	Lower quartile	Median	Upper quartile	Maximum
Age *years*	61.9	10.1	39	54	64	70.2	84
Age at diabetes onset *years*	41.3	12.7	10	33	40	50.0	82
Diabetes duration *years*	20.6	9.4	2	14.3	20.0	28.0	43.0
Pre-HD [CP] *nmol/L*	3.22	1.85	0.19	2.07	2.83	4.24	8.81

Abbreviations are: HD, hemodialysis; [CP], C-peptide concentration.

If diabetes onset appeared when patients were less than 30 years of age, they were classified as type 1 diabetics if stimulated C-peptide concentrations were ≥20 nmol/L but ≥0.50 nmol/L, and patients were classified as type 2 diabetics if the stimulated C-peptide levels were >0.50 nmol/L.

Statistics

All analyses were performed using a C-STAT® package (Oxford Statistics, UK). Intergroup differences between continuous variables were assessed by two-tailed, nonpaired *t*-tests and analysis of variance (ANOVA; multiple comparisons). Significant differences in proportions were assessed by chi-square test. $P < 0.05$ was considered to be significant.

RESULTS

Study population demographics

One hundred twenty-seven ESRD diabetic patients (48% males) were studied: 75 (59.1%) African Americans (AA), 46 (36.2%) Caucasians (CA), and 6 (4.7%) Hispanics (H). Age, age at diabetes onset, diabetes duration, and mean C-peptide concentrations are presented in Table 2. Demographic parameters (age and gender distribution) or diabetes profile (diabetes duration, age at diagnosis, C-peptide levels, diabetes treatment, and body mass index) were similar in the three ethnic groups (data not shown). Diabetic ESRD patients ($N = 79$, 62.2%) treated with insulin at the time of the study were significantly more obese, had less optimally controlled serum glucose, and had lower C-peptide levels as compared with those ($N = 48$) treated by only diet and/or oral hypoglycemic agents (Table 3).

Table 3. Comparison between patients treated with insulin at the time of the study and those on diet and/or oral hypoglycemic agents

	Current therapy for diabetes		
	Insulin ($N = 79$)	Diet and/or oral hypoglycemic agents ($N = 48$)	*P*
% Males	20.3	64.5	<0.05
% African Americans	55.7	62.5	NS
Insulin treatment at diagnosis *% of patients*	45.6	18.8	<0.05
Diabetes phenotype[a]			NS
Type 1	12	1	
Type 2	49	39	
Type unknown	18	9	
Pre-HD [CP] *nmol/L*	2.90 ± 1.78	3.74 ± 1.86	<0.05
[Glucose] *mg/dL*	196.3 ± 100.1	129.8 ± 70.9	<0.05
Age at diagnosis *years*	39.0 ± 12.1	44.9 ± 13.1	<0.05
Diabetes duration *years*	21.6 ± 19.3	19.0 ± 9.3	NS
BMI at 18 years kg/m^2	26.8 ± 8.6	25.8 ± 7.0	NS
Maximum BMI kg/m^2	38.1 ± 10.6	36.3 ± 8.5	NS
Current BMI kg/m^2	30.9 ± 8.2	26.9 ± 4.9	<0.05

Abbreviations are: HD, hemodialysis; [CP], C-peptide concentration; [glucose], glucose concentration; BMI, body mass index.
[a] Based on clinical criteria defined in the **Methods** section

Classification of ESRD diabetic patients according to clinical criteria

Using clinical phenotyping criteria (**Methods** section), we could categorize only 101 (79.5%) of the 127 ESRD diabetic patients: 13 were type 1 (10.2%) and 88 were type 2 (69.3%) diabetics. Type 1 and type 2 diabetic ESRD patients had similar demographic profiles [age, ethnic distribution, and gender distribution (data not shown)], except for diabetes duration (17.9 ± 8.7 vs. 29.9 ± 5.9 years for type 2 vs. type 1 diabetic, respectively, $P < 0.001$). Twenty-six patients could not be categorized as type 1 or type 2 diabetics. Of these, 10 patients were treated only with insulin (for a mean period of 24.1 ± 8.2 years; minimum period of treatment was 9 years), but had onset of diabetes between 30 and 38 years of age. The remaining 16 patients, who could not be classified using clinical information, were treated initially with oral hypoglycemic agents, but had an onset of diabetes before 35 years of age (8 patients were 30 or younger at diagnosis) and required insulin therapy within two years after initial diagnosis. None of these 26 patients had a history of DKA. Clinical phenotyping results are summarized in Table 4.

Classification of ESRD diabetic patients using serum C-peptide levels

C-peptide concentrations for the study population are presented in Table 2. As shown in Figure 2, contemporaneous C peptide and glucose concentrations failed to correlate significantly. In addition, C-peptide levels did

Table 4. Clinical classification of diabetes phenotypes in 127 diabetic ESRD patients

Standard clinical criteria[a]					
	Unclassifiable diabetes type (N = 26)				
Type 1 (N = 13)	Revised clinical criteria[b]				Type 2 (N = 88)
	Insulin as only treatment (N = 10) Age at onset		Insulin initiated 1 to 2 years after DM onset (N = 16) Age at onset		
	<35 (N = 4)	≥35 (N = 6)	≤30 (N = 9)	>30 (N = 7)	
Certain type 1 DM	Consistent with type 1 DM	Unclassifiable DM		Consistent with type 2 DM	Certain type 2 DM
Type 1 DM (N = 17)		MODY or Early onset type 2 (N = 15)		Type 2 DM (N = 95)	

Abbreviations are: DM, diabetes mellitus; ESRD, end stage renal disease; MODY, maturity onset diabetes of the young (monogenic diabetes).

[a] See **Methods** for details

[b] Less stringent clinical parameters that classified diabetic ESRD patients, treated only with insulin and with diabetes onset <35 years of age, as type 1 diabetics and those who were 30 to 40 years of age and not treated with insulin use during the first year after diabetes onset, as type 2 diabetics. See **Discussion** for details.

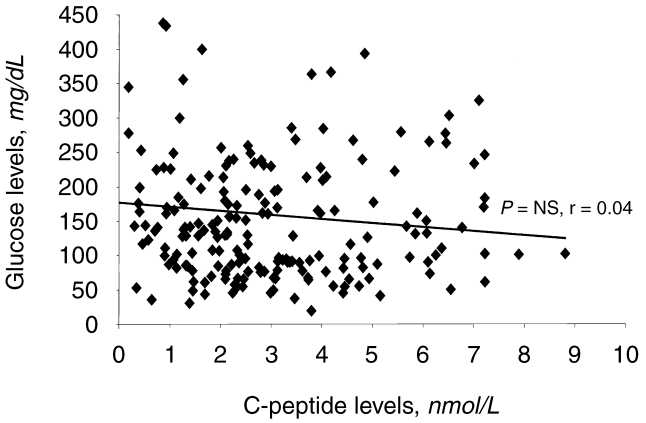

Fig. 2. Relationship between serum glucose and C-peptide levels in 127 diabetic ESRD patients. Scatter plot with each data point representing contemporaneous C-peptide and plasma glucose concentrations. The data were subjected to linear regression analysis, with the best fit line depicted. Statistical significance is defined as $P < 0.05$; P = NS, r = 0.04.

not correlate with the age of diabetes onset, diabetes duration, or body mass indexes (data not shown).

Of the 127 ESRD diabetic patients in the study, 122 had pre-hemodialysis C-peptide levels >0.50 nmol/L. Mean corresponding glucose concentrations were 170.6 ± 107.7 mg/dL, with 44 subjects having a serum glucose level ≤120 mg/dL. All 122 patients (group A) were classified as type 2 diabetics using the algorithm described in the **Methods** section and in Figure 1. Although three ESRD patients (group B) had pre-hemodialysis C-peptide concentrations between 0.20 and 0.50 nmol/L, they were classified as type 2 diabetics (Fig. 1) since their ages at diabetes onset were 36, 37, and 40 years. The remaining two patients (group C) had pre-hemodialysis C-peptide concentrations <0.20 nmol/L (0.6 ng/mL) despite concomitant glucose levels of 278 and 375 mg/dL. These two patients were classified as type 1 diabetics since stimulated C-peptide concentrations remained <0.20 nmol/L.

Comparison of classification of diabetic ESRD patients using clinical and C-peptide phenotyping criteria

Classifications of diabetic ESRD patients, using clinical or C-peptide criteria are compared in Table 5. Clinical and C-peptide–based classifications of diabetes were concordant in only 70.1% of all cases. Eleven of 122 (9.0%) diabetic ESRD patients with significantly elevated (>0.50 nmol/L) C-peptide levels were type 1 diabetics, using standard clinical criteria (age of diabetes onset <25 years, treatment only with insulin and/or history of DKA). Surprisingly, diabetic ESRD patients, classified as type 1 (N = 11) or type 2 (N = 88) using clinical criteria, had nearly identical mean C-peptide concentrations (3.08 ± 1.99 vs. 3.37 ± 1.81 nmol/L, respectively, P = NS). Using clinical criteria as the standard, sensitivity, specificity, positive, and negative predictive values of C-peptide concentrations in classifying diabetic ESRD patients are presented in Table 6.

C-peptide levels in nondiabetic ESRD patients

Since the kidney is the major site of C-peptide metabolism and excretion, we determined pre-hemodialysis C-peptide concentrations in 30 nondiabetic ESRD patients (diabetes excluded by normal HbA_{1c} values, normal glucose concentrations, and medical history) who were matched for gender, race, and dialysis duration with the study group population. C-peptide concentrations were also assayed in diabetic, first-degree relatives with normal serum creatinine levels (N = 37). Distributions of C-peptide concentrations in diabetic ESRD patients, nondiabetic ESRD patients, and first-degree relatives are shown in Figure 3. C-peptide concentrations were similar in diabetic and nondiabetic ESRD patients, but were 2.5 to 2.7 times higher compared with diabetic siblings with normal renal function. The percentages of type 1 and type 2 diabetics, according to clinical criteria, were similar in the diabetic ESRD patients and their first-degree relatives. These data suggest that C-peptide

Table 5. Comparison between classification of diabetic ESRD patients using algorithms based on clinical and C-peptide concentrations

C-peptide range[a]	Group A ($N = 122$) *>0.50 nmol/L*			Group B ($N = 3$) *0.20–0.50 nmol/L*		Group C ($N = 2$) *<0.20 nmol/L*
DM classification by [CP]	Type 2			Type 2		Type 1
DM classification by clinical parameters	Type 1 ($N = 11$)	Type uncertain ($N = 25$)	Type 2 ($N = 86$)	Type 2 ($N = 2$)	Type uncertain ($N = 1$)	Type 1 ($N = 2$)
CP levels (mean ± SD)	3.08 ± 1.97	3.35 ± 1.69	3.37 ± 1.81			
[range]	[0.52–6.07]	[0.73–6.52]	[0.76–8.81]	[0.44 and 0.46]	[0.34]	[0.19 and 0.19]

Abbreviations are: DM, diabetes mellitus; CP, C-peptide concentrations, nmol/L; DCCT, Diabetes Complications Control Trial.
[a] Study group was stratified using the DCCT study group algorithm (see **Methods** and **Figure 1**)

Table 6. Sensitivity, specificity, positive predictive and negative predictive values of different C-peptide derived algorithms for correct identification of type 2 diabetic ESRD patients

Comparison	Se	Sp	PPV	NPV
Classification based on [CP] (see **Methods** and **Figure 1**)				
1/ vs. accepted clinical criteria (see **Methods**)	100%	5.1%	70.4%	100%
2/ vs. revised clinical criteria[a]	100%	6.3%	76.0%	100%
Pre-HD [CP] >0.50 nmol/L (no age of DM onset criteria is used)				
1/ vs. strict clinical criteria for reference	97.7%	7.7%	70.5%	60%
2/ vs. revised clinical criteria[a]	97.8%	9.4%	76.2%	60%
New algorithm to identify ESRD patients as type 2 DM: pre-HD [CP] >0.50 nmol/L and age of DM onset ≥38 years	87.2%	95.1%	97.4%	78.0%

Abbreviations are: HD, hemodialysis; DM, diabetes mellitus; ESRD, end-stage renal disease; [CP], C-peptide concentration; DCCT, Diabetes Complications Control Trial; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.
[a] Legend of Table 3 and **Discussion** for definition

concentrations poorly reflect pancreatic insulin synthesis once diabetic patients have ESRD.

DISCUSSION

As part of a study to identify genes that regulate diabetic nephropathy, the accuracy of clinical and biochemical phenotyping criteria to classify diabetes was evaluated in ESRD patients [36]. A precise phenotype is necessary to identify genetic loci [15], but phenotyping criteria for type 1 and type 2 diabetes are not well validated in the ESRD population and are imprecise. For example, patients with diabetes onset from age 25 to 30 years are not included in any of the published classification schemes, which we used to develop clinical phenotyping criteria for ESRD patients. Further confounding classification, a significant proportion of diabetic patients have indeterminate phenotypes with features of both type 1 and type 2 diabetes, and diabetes onset may occur at least four to seven years before diagnosis in type 2 diabetics [37]. We found that multiple clinical criteria were unable to categorize a significant proportion (21%) of a diabetic ESRD population as either type 1 or type 2 diabetics. To improve the discriminating power of our clinical criteria, we added C-peptide concentrations, a sensitive and specific parameter that distinguishes type 1 from type 2 diabetic patients [1–3, 21, 25, 29, 31, 38–41]. However, our data show that C-peptide concentrations did not improve the power of clinical criteria to classify diabetic ESRD patients accurately. C-peptide concentrations misclassified as type 2 diabetics a substantial proportion of ESRD diabetic subjects who were classified as type 1 diabetics when using accepted clinical parameters. When clinical criteria are considered as the standard, the specificity of C-peptide concentrations to distinguish type 1 from type 2 diabetes, using norms derived from diabetics with normal renal function (DCCT study) [2], is only 5% when applied to diabetic ESRD patients. C-peptide levels in type 2 diabetic ESRD patients were indistinguishable from type 1 ESRD diabetics or from nondiabetic ESRD individuals. However, the number of type 1 diabetics enrolled in this study is small, and a larger sample size may have improved the power of C-peptide concentrations to discriminate type 1 and type 2 diabetic ESRD patients.

To classify patients with type 1 and type 2 diabetes better, biochemical assays based on pathogenetic mechanisms have been developed to identify an autoimmune response directed against pancreatic β cells, that is, anti-islet cell or antiglutamic acid decarboxylase antibodies [1, 5, 42] or functional insulin secretory deficiency, that is, C-peptide determinations. Several studies have shown that in populations with normal renal function, C-peptide concentrations discriminate type 1 from type 2 diabetes

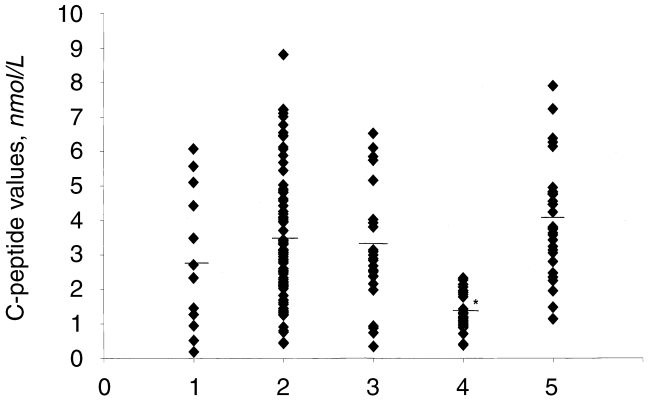

Fig. 3. Distribution of the pre-hemodialysis C-peptide concentrations in the diabetic ESRD study population, their diabetic siblings with normal renal function and nondiabetic ESRD patients. 1 = diabetic ESRD patients classified as type 1 diabetes based on clinical criteria (N = 13); 2 = diabetic ESRD patients classified as type 2 diabetes based on clinical criteria (N = 88); 3 = diabetic ESRD patients who could not be classified by the clinical criteria (N = 26); 4 = diabetic patients with normal renal function (control group 1); 5 = nondiabetic ESRD patients (control group 2). The clinical classification algorithm is described in the **Methods** section (*$P < 0.05$).

[2, 3, 31] and may be used to select the initial therapy [38] and predict future insulin requirements [39]. The discriminative value of C-peptide concentrations increases with diabetes duration. Five years after diagnosis, all 610 type 1 diabetics included in the DCCT study had fasting C-peptide concentrations <0.20 nmol/L [2]. In contrast, no impairment of β-cell function was described in type 2 subjects, who lack anti-islet cell antibodies [43]. C-peptide concentrations are routinely assayed after fasting or in a provocative test. Some studies have suggested that a stimulated value adds little information [31, 44, 45], but other authors emphasize the importance of changes in C-peptide levels following stimulation with glucagon or a standardized meal [31, 38, 39]. In contrast to most studies, we wished to document ongoing insulin synthesis in diabetic ESRD patients by measuring pre-hemodialysis C-peptide concentrations. Fasting and stimulated C-peptide levels were obtained only for clarification in a few patients (1.5%) with low (<0.5 nmol/L) C-peptide values and an early age of diabetes onset (**Methods** section).

Previous studies have classified diabetic phenotypes within an ESRD population. Cowie et al developed an extensive, four-stage classification based on clinical criteria to distinguish between type 1 and type 2 diabetes [29]. Similar to our results, 20% of their study population did not fit the clinical criteria for either type 1 or type 2 diabetes. Classification was clarified only after the clinical data for each individual were scored, and four of six diabetologists agreed. Despite these efforts, 9% of ESRD patients remained unclassified.

Benhamou et al classified diabetic ESRD patients (N = 88, a subset of the UREMIDIAB population [30]) with a single fasting C-peptide measurement using DCCT study criteria [21]. The positive predictive values of C-peptide concentrations <0.20 nmol/L was 100% for type 1 diabetes and 96% for C-peptide concentrations >0.20 nmol/L for type 2 diabetes. In contrast to our results, the concordance between the C-peptide concentrations and the clinical classification, using criteria similar to ours, in the UREMDIAB diabetic ESRD population was excellent. Clinical criteria categorized only 4% of patients with measurable C-peptide concentrations as type 1 diabetics. Our results may be discrepant for two reasons. First, the racial composition of study populations was dissimilar. Ninety percent of the UREMDIAB subjects were Caucasians [21] compared with only 39% in our study. Race may be an important variable in estimating the prevalence of type 1 and type 2 diabetes from clinical criteria [29, 41]. To address this possibility, we applied clinical criteria (fasting C-peptide limit of ≥0.3 nmol/L, age of onset ≥28.9 years, and body mass index ≥31.7) for type 2 diabetes used in a large study population (N = 3694) composed of 80% African Americans [41]. With re-analysis using these revised criteria, 11.2% of type 1 diabetic ESRD patients in our study population remain misclassified by C-peptide concentrations, with little improvement in the concordance between classifications based on clinical criteria and C-peptide concentration stratification. A second difference between the UREMDIAB analyses and ours was that individualized criteria were used to categorize diabetic ESRD patients in the UREMIDIAB study [21], an approach that may have improved agreement between clinical and C-peptide concentration classification schemes. To test whether the concordance between the classification schemes would improve with a similar approach, we also re-analyzed, using revised clinical parameters, 26 patients who could not be classified as either type 1 or type 2 diabetes (Table 4). Patients treated with only insulin and with a diabetes onset at <35 years of age (N = 4) were categorized as type 1 diabetics. Patients with age of onset between 30 and 40 years of age were classified as type 2 diabetics if insulin therapy was not instituted during the year after diabetes diagnosis (N = 7). Despite using these less stringent criteria, 15 diabetic ESRD subjects remained unclassified (Table 4), and concordance between the classification algorithms only marginally improved (from 70% to 75%). In addition, the proportion of type 1 diabetic ESRD subjects misclassified by C-peptide concentration criteria also increased (from 9.0% to 12.3%) when the revised clinical criteria were applied.

Other studies have assayed C-peptide concentrations in diabetic and nondiabetic patients, with renal insufficiency. C-peptide concentrations were elevated approximately fourfold in diabetic ESRD patients compared with diabetics with normal GFR [40, 46], consistent with

our observations. C-peptide concentrations also have been shown to be elevated in nondiabetic animals [47] and patients [22, 23] with renal insufficiency. Mean fasting C-peptide concentrations in nondiabetic continuous ambulatory peritoneal dialysis patients have been reported to be 4.3 nmol/L as a result of increased proinsulin synthesis stimulated by the peritoneal glucose load [35]. Regeur, Faber, and Binder reported that the mean fasting C-peptide level in anephric, nondiabetic patients was 2.20 nmol/L (range of 1.60 to 3.28 nmol/L), which was six times higher than in controls with normal renal function (range of 0.18 to 0.63 nmol/L) [23], and similar to prehemodialysis values measured in nonfasting, nondiabetic, ESRD patients by others [48]. Our nondiabetic hemodialysis patients also had elevated C-peptide concentrations, which were similar to those measured in our diabetic ESRD study population and significantly higher than diabetic siblings with normal renal function. Based on published evidence and our data, we conclude that C-peptide concentrations alone cannot discriminate type 2 from type 1 diabetes in ESRD patients, since C-peptide or immunoreactive C-peptide fragments accumulate in this patient population.

A new algorithm, using C-peptide concentrations and age, to classify diabetic ESRD patients

Given the overlap of C-peptide concentrations measured in type 1 diabetic, type 2 diabetic, and nondiabetic ESRD patients, merely changing the stratification algorithm to classify diabetic ESRD patients would not improve its discriminative power. In our study, C-peptide concentrations exceed the median C-peptide level from type 2 diabetic ESRD patients in 53% of type 1 and unclassified ESRD diabetics. However, our data do suggest a new classification algorithm, which will need to be tested in prospective studies. Patients in our group A cohort (C peptide >0.50 nmol/L) misclassified by C-peptide concentrations included 11 type 1 diabetics and 25 unclassified subjects (Table 5). All 11 type 1 diabetic patients had diabetes onset at <25 years of age, and the maximum age of diabetes onset was <38 years for the ESRD patients who could not be classified by accepted clinical criteria. These observations suggest a C-peptide concentration >0.50 nmol/L in an ESRD patient with diabetes onset after 38 years would accurately classify 87% of the ESRD diabetic patients, with an 97.4% positive predictive value, using clinical criteria as a standard (Table 6). Since this algorithm requires only a random C-peptide value and age of diabetes onset, rather than more extensive medical history, the proposed model could easily be utilized in epidemiological studies.

In conclusion, neither clinical criteria nor C-peptide measurements can independently and precisely classify all diabetic ESRD patients, using norms established in subjects with normal GFR. Approximately 85% of diabetic ESRD patients in our study who were classified as type 1 diabetics using accepted clinical criteria had C-peptide values indistinguishable from type 2 diabetics. Accurate classification of diabetic ESRD patients for genetic epidemiological studies will require both clinical and biochemical criteria, which would need to be validated in dialysis populations.

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